ANAPLEROTIC THERAPY IN

PROPIONIC ACIDEMIA

by

Leisa Bitner Price

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Nutrition

College of Health

The University of Utah

May 2013

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The following faculty members served as the supervisory committee chair and members for the thesis of___Leisa Bitner Price_____.

Dates at right indicate the members' approval of the thesis.

_____E. Wayne Askew_____, Chair	_02/26/2013_____ Date Approved
_____Nicola Longo_____, Member	_02/26/2013_____ Date Approved
_____Lisa Joss-Moore_____, Member	_02/26/2013_____ Date Approved

The thesis has also been approved by E. Wayne Askew , Chair of the

Department/School/College of Nutrition

and by Donna M. Wight, Interim Dean of The Graduate School.

ABSTRACT

Propionic acidemia is a rare metabolic disorder caused by a deficiency of propionyl- CoA carboxylase, the enzyme that converts propionyl-CoA to methylmalonyl-CoA. Patients with propionic acidemia cannot metabolize propionic acid, which sequesters oxaloacetate from the Krebs cycle to form methylcitric acid. This may lead to a deficiency in Krebs cycle intermediates. Our study looked at whether adding glutamate, citrate, or ornithine α-ketoglutarate (chemicals that could fill up the Krebs cycle as anaplerotic agents) to patients' diets affected plasma levels of glutamine and ammonia, and the urinary excretion of Krebs cycle intermediates. We also looked at developmental markers and hospitalizations to determine clinical efficacy. Each supplement was administered daily for four weeks with a two week washout period between supplements. The supplement that produced the most favorable changes was supplemented for 30 weeks following the initial study period. Levels of Krebs cycle intermediates, α-ketoglutarate, succinate, and fumarate increased significantly compared to baseline during citrate supplementation. Glutamine levels did not decrease with increasing ammonia levels. Glutamate and alanine levels significantly increased, rather than decreased with increasing ammonia levels. There was a significant direct correlation between lysine and ammonia. Hospitalizations per year decreased significantly in the 2 years following the study compared to the 2 years before and

during the study. These results indicate that citrate entered the Krebs cycle providing successful anaplerotic therapy by increasing levels of the downstream intermediates of the Krebs cycle: α-ketoglutarate, succinate and fumarate. Citrate supplements appear to be a safe and might have contributed to reducing hospitalizations in patients with propionic acidemia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to acknowledge the help of my thesis committee, Dr. Askew, Dr. Longo, and Dr. Joss-Moore, for their patience and flexibility in helping me complete my thesis. I would also like to acknowledge Sharon Ernst, Krista Viau, and Marilyn Knuth for teaching me the ins and outs of dietary management in patients with metabolic diseases.

I would especially like to acknowledge the help and support from my husband, Coby Price, and my extremely supportive family. They stood with me through the entire process and I would have never been able to complete my thesis or degree without them.

INTRODUCTION

Propionic acidemia is an autosomal recessive disorder caused by deficiency of propionyl- CoA carboxylase, the enzyme that converts propionyl-CoA to methylmalonyl-CoA (Figure 1) using the cofactor biotin (1). This mitochondrial pathway is essential for degradation of the amino acids isoleucine, methionine, threonine, and valine, odd chain fatty acids, and cholesterol (1). Propionic acid also originates from the catabolism of the nucleotides thymine and uracil and from bacterial production of propionate from pyruvate in the gut (1). Propionyl-CoA is eventually converted into succinyl-CoA and enters the citric acid (Krebs) cycle for energy production. In propionic acidemia, excess propionyl-CoA combines with oxaloacetate, an intermediate of the Krebs cycle, to form methylcitric acid, the diagnostic metabolite of propionic acidemia.

Most cases of propionic acidemia present with lethargy progressing to coma from 16 hours to weeks after birth, depending on the severity of the enzyme impairment caused by the genetic lesion (1). Coma is caused by metabolic imbalance and severe hyperammonemia that can be associated, or not, with metabolic acidosis (2). Even when patients are rescued from the hyperammonemic coma, the prognosis is poor since patients can develop life-threatening complications such as pancreatitis or cardiomyopathy (3, 4). These complications, whose mechanism is unknown, cause severe morbidity and

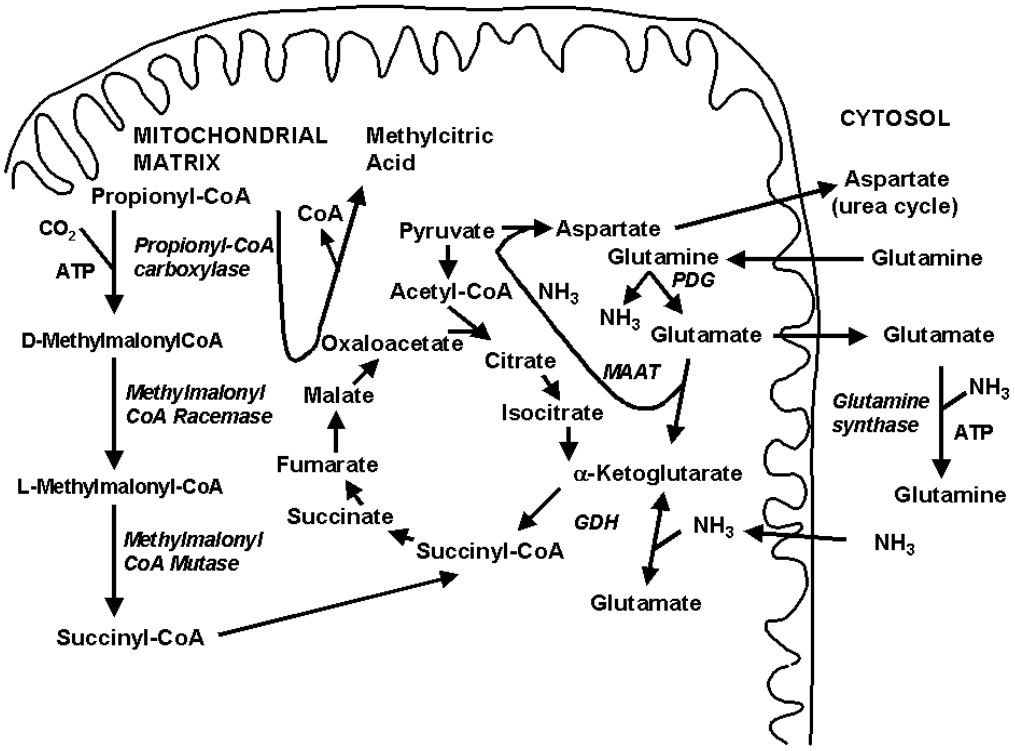

Figure 1. Propionyl-CoA and glutamine metabolism in propionic acidemia. Propionyl-CoA is carboxylated by propionyl CoA carboxylase to become D-methylalonyl-CoA. With the action of methymalonyl CoA racemase and mutase, this produces succinyl-CoA that fills up the Krebs cycle. In propionic acidemia, propionyl-CoA accumulates and condenses with oxaloacetate to produce methylcitric acid. The decrease in oxaloacetate and succinyl-CoA can impair the Krebs cycle, reducing the concentration of α-ketoglutarate. This can be repleted to the expense of glutamine and glutamate. *GDH: Glutamate dehydrogenase; MAAT: mitochondrial aspartate amino transferase; PDG: phosphate-dependent glutaminase (12).*

mortality even in optimally treated patients limiting the benefits of early diagnosis by newborn screening programs (3, 4).

Ammonia accumulation in organic acidemias can be secondary to inhibition of carbamylphosphate synthase-1 (CPS-1), the first enzyme of the urea cycle. In fact, propionyl-CoA competitively inhibits the synthesis of N-acetylglutamate (5), an allosteric activator of CPS-1 whose levels are reduced in livers of rats receiving propionic or methylmalonic acid (6), and N-carbamylglutamate, a precursor of N-acetylglutamate, reduces plasma ammonia levels in patients with propionic or methylmalonic acidemia (7, 8). Chronic hyperammonemia in propionic acidemia, however, differs from that of urea cycle defects, since it is not accompanied by an increase in glutamine, the amino acid that shuttles nitrogen groups to the liver for ammonia formation and detoxification (9, 2, 10).

Ammonia is generated from amino acid metabolism within the absorptive cells of the intestine, from bacterial hydrolysis of urea in the colon, from catabolism of branched chain amino acids in peripheral tissues (muscle) with generation of alanine and glutamine, and from the catabolism of all amino acids within the liver. Most of the ammonia arrives to the liver from the portal vein which has an ammonia concentration higher than that of the systemic circulation. In periportal hepatocytes, the urea cycle is extremely active. The ammonia not converted to urea by periportal hepatocytes can be conjugated with glutamate to form glutamine by the high-affinity glutamine synthase in centrolobular hepatocytes (11). In urea cycle defects, the excess ammonia reaching centrolobular hepatocytes results in extremely elevated glutamine levels.

In propionic acidemia, glutamine levels are low even in well-controlled patients and decrease (rather than increase) with hyperammonemia (12). We have proposed that

this glutamine paradox, also seen by other groups (9, 2, 10), could be due to a dysfunctional Krebs cycle, with deficiency of α-ketoglutarate (12). In propionic acidemia, glutamate dehydrogenase, normally a cataplerotic enzyme favoring the exit of α-ketoglutarate as glutamate (13), works in reverse generating α-ketoglutarate from glutamate (12). Low levels of glutamate favor the release of ammonia from glutamine to generate glutamate, explaining the association between high ammonia and low glutamine levels.

Propionic acid is important in the anaplerosis (filling-up) of the Krebs cycle as indicated by the clinical improvement of patients with fatty acid oxidation disorders and pyruvate carboxylase deficiency treated with heptanoin, an odd-chain fatty acid that is metabolized to propionic acid and converted to succinyl-CoA (14, 15). Since such a mechanism is so effective in replenishing the Krebs cycle, its complete absence in patients with propionic acidemia, coupled with the sequestration of oxaloacetate by propionyl-CoA to form methylcitrate, should result in a severe deficiency of all intermediates of the citric acid cycle. In muscle, α-ketoglutarate is the intermediate with the lowest concentration (0.05 mmol/kg) after oxaloacetate (0.012 mmol/kg, (13, 16)). α-Ketoglutarate concentration further declines with exercise (13) and is regenerated from glutamine and glutamate (13, 16). If glutamine/glutamate and α-ketoglutarate are too low, such as in propionic acidemia (12), the process might become ineffective in generating energy, possibly contributing to hypotonia and organ failure.

Supplements can provide substrates to the Krebs cycle. In patients with argininosuccinic aciduria, citrate, the intermediate with the highest concentration in the Krebs cycle (0.362 mmol/kg in muscle, (13, 17)), can generate cytoplasmic aspartate to

increase conjugation with citrulline and the urinary excretion of the water-soluble argininosuccinic acid (18, 19). This action does not require entry of citrate into mitochondria and this therapy is not routinely used given its modest efficacy and possible side effects (metabolic alkalosis, (19)). Citrate in combination with aspartate stabilized the metabolic control of one patient with pyruvate carboxylase deficiency (20) and increased plasma glutamine levels in another patient (14). Both patients had severe neurological compromise and citrate effects on the overall outcome were difficult to assess given the concomitant use of other medications/supplements (14, 20).

Other natural supplements include glutamate, glutamine, and α-ketoglutarate. Glutamate is mostly (>90%) metabolized by the splanchnic bed, while more than 50% of oral glutamine can reach the circulation and increase glutamate production (21). Glutamine has been used extensively in the treatment of very low birth weight infants in whom it is extremely safe (up to 0.6 g/kg/day) and prevents protein catabolism (22). Glutamine (2 g/m^2 BID) decreases narcotic requirements and the number of days of intravenous hyperalimentation in cancer patients receiving chemotherapy (23). α-Ketoglutarate is available as a salt or in combination with the positively charged amino acids arginine and ornithine. The salt form and arginine α-ketoglutarate are widely available as supplements, but only limited clinical trials have been reported (24). Ornithine α-ketoglutarate has been used since the early 1960s and is more effective than α-ketoglutarate or ornithine alone in increasing glutamine levels in healthy subjects (24). This is because ornithine can be converted into glutamate through ornithine amino transferase and Δ^1pyrroline-5-carboxylate dehydrogenase (Figure 2) (24, 25). Ornithine α-ketoglutarate improves the nutritional status in patients with burns and in children

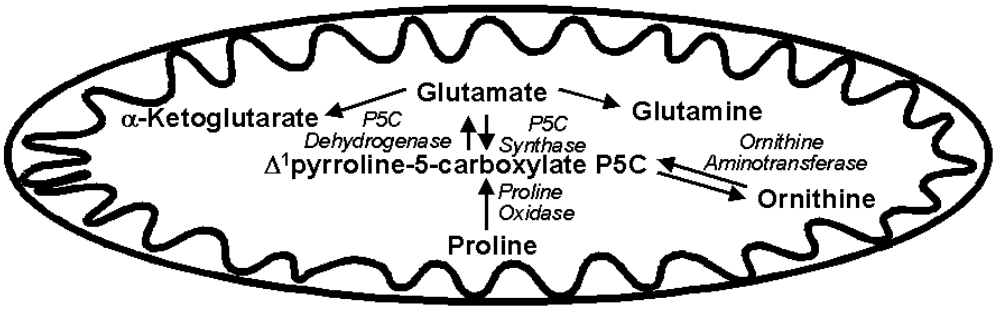

Figure 2. Selected pathways of ornithine metabolism.
Ornithine can be converted to glutamate by the action of two enzymes, ornithine amino transferase and Δ1pyrroline-5-carboxylate dehydrogenase

receiving total parental nutrition (26, 27). The combination of ornithine and α-ketoglutarate can spare endogenous glutamine (increasing its plasma levels), feed directly into the Krebs cycle, and participate in the synthesis of urea cycle amino acids (25).

Since in propionic acidemia there is a direct correlation between plasma levels of glutamine/glutamate and levels of the urea cycle amino acids ornithine and arginine (12), the simultaneous administration of ornithine and α-ketoglutarate should have a synergistic effect. Both glutamine and ornithine α-ketoglutarate are extremely well tolerated in humans at doses up to 0.6 g/kg/day and have not caused any known adverse reaction. They are freely available as nutritional supplements to the general public and are marketed mostly as daily supplements for body builders or for general health.

A mouse model of propionic acidemia has been generated by disrupting the gene encoding the α subunit of the enzyme (28). Affected mice die at birth by acute ketoacidosis, but can be rescued by gene therapy (28). In the initial experiments, all animals died even with this therapy since it was difficult to obtain the appropriate gene dosage to allow survival (28). More recently, gene therapy with adeno-associated virus serotype 8 gene transfer was effective in rescuing the neonatal lethal phenotype of mice with propionic acidemia (34). It remains a formidable task to study in these mice the effects not only of a restricted diet, but also of dietary supplements.

Our hypothesis is that functional insufficiency of the citric acid (Krebs) cycle in propionic acidemia decreases production of α-ketoglutarate that is replenished by glutamine/glutamate. Decreased availability of glutamate/glutamine explains the chronic hyperammonemia of patients with propionic acidemia while decreased ATP production could be responsible for the low muscle tone and progressive organ dysfunction. To test this hypothesis, anaplerotic supplements (citrate, glutamine, or ornithine α-ketoglutarate) were administered to patients with propionic acidemia to evaluate their effects on plasma levels of glutamine and ammonia, and the urinary excretion of Krebs cycle intermediates (to determine whether the supplements entered mitochondria). In addition, the clinical efficacy of these supplements was tested by looking at mental and physical development and hospital admissions. This therapy would be relatively easy for these patients (supplements can be added to their special formula), inexpensive, and could potentially lead to great benefit by preventing one of the major causes (hyperammonemia) of mental deterioration and death. More importantly, a dysfunction of the Krebs cycle with subsequent decreased energy production could also explain other signs and symptoms

(hypotonia, organ failure) of patients even with mild variants of propionic acidemia. This therapy, if effective in the small number of patients tested in this project, could be extended to patients with other organic acidemias (methylmalonic acidemia and possibly others) with a larger, multicenter clinical trial, required to enroll a sufficient number of patients with propionic and other types of organic acidemias.

METHODS

Three patients with propionic acidemia followed at the University of Utah since birth were enrolled in this study. Their ages at time of enrollment ranged from 5.9 to 11 years of age. Their developmental quotient/IQ ranged from moderately to severely delayed with the gross motor area being the most affected. Each patient in the 2 years prior to the trial required two to six hospital admissions per year to control metabolic decompensation or intercurrent illnesses. Their metabolic control was typically excellent.

Prior to beginning and at the conclusion of the study, each patient received a cognitive and motor evaluation. The cognitive evaluation was completed by a licensed psychologist (Dr. Nancy Cantor) using the Stanford-Binet Intelligence Scales (SB5), Fifth Edition. The motor activity assessment was performed by a licensed physical therapist (Dr. Eduard Gappmaier) and included hand-held myometry studies, time-function tests, and an accelerometry evaluation.

The study occurred in two parts. In the first part, individual supplements (ornithine α-ketoglutarate, glutamine, and citrate) were evaluated for their ability to raise plasma glutamine levels, or to cause other biochemical changes as compared to baseline (before the study and during wash-out periods). In the second part, the diet of the patients was supplemented with the supplement judged to produce the most favorable changes (citrate) for 30 weeks. Doses of supplements (ornithine α-ketoglutarate, glutamine, and citrate) were based on patients' weights at weeks 0, 6, 12, and 18 and

were not changed during the corresponding treatment period (4 weeks or 30 weeks). Ornithine α-ketoglutarate 400 mg/kg (2 mmol/kg ornithine, 1 mmol/kg α-ketoglutarate) per day was started at week 0 and continued until week 4. Week 4-6 was a washout period with no supplements for 2 weeks. Glutamine 400 mg/kg (2.74 mmol/kg) per day was started at week 6 and continued through week 10. A second washout period occurred from week 10-12 with no supplements for 2 weeks. At week 12, citrate (7.5 mEq/kg, 2.5 mmol/kg) was started and continued through week 16. The final washout period occurred from week 14-16. At week 18, citrate was started at the same dosing level and continued through week 48 when measurements were repeated, the study was officially concluded, and patients were offered to continue citrate supplements on a clinical basis. All patients continued citrate supplements for at least 2 years following the conclusion of the study.

Study visits occurred each week starting at baseline and continued until week 16, with the exception of two week washout periods. During the final treatment period, week 18-48, study visits occurred every 6 weeks. Each study visit included a physical exam along with anthropometric and vital sign measurements. Diet records were collected and analyzed during the final visit of each trial period (weeks 4, 10, 16, 48). Concomitant medications and therapies were collected and recorded.

After the initial evaluation, parents were given the nutritional supplement to mix in the child's metabolic formula containing all amino acids except propiogenic amino acids (isoleucine, methionine, threonine and valine). Patients were also receiving a measured amount of natural proteins from regular food. Following the initial study period, lab results were drawn periodically at clinic visits and were analyzed for long-

term alterations in amino acid levels and plasma ammonia levels. Data were collected through a retrospective chart review.

Study labs included plasma amino acids, acylcarnitine profile, ammonia, lactic acid, and urine organic acids. Biochemical tests were performed at ARUP laboratories of the University of Utah. Plasma amino acids were analyzed by ion-exchange chromatography with post-column ninhydrin detection (Biochrom 30 amino acid analyzer) (29). Urinary organic acids were analyzed by gas chromatography-mass spectrometry (HP/Agilent) (30). Plasma acylcarnitine levels and profile were determined by tandem mass spectrometry (Waters Quattro Premiere) using stable isotope standards for quantitation. Dr. Marzia Pasquali (ARUP) quantified analytes not routinely reported (methylcitrate, other propionic acid metabolites in urine, propionylcarnitine in plasma), performed subsequent data analysis, and oversaw consistency of measurements among different samples.

Plasma amino acids were compared before and after treatment with anaplerotic agents. Baseline study labs (week 0, 6, 12, and 18) (ammonia, amino acids, organic acids, lactic acid) were averaged and compared to those obtained during treatment with ornithine α-ketoglutarate (n=4), glutamine (n=4) and citrate (n=4). Data were compared using analysis of variance with $p<0.05$ as a statistical cutoff. Patients were initially tested against themselves and subsequently data were pooled together to determine if any significant differences occurred. Correlation analysis was also used to compare plasma ammonia levels to glutamine levels. Calculations were performed using Microsoft Excel and SigmaPlot software, version 8.0.

RESULTS

Patients and Safety

Table 1 summarizes the clinical and anthropometric data of patients with propionic acidemia. Three patients participated in this study (age at the beginning of the study ranged from 5.9 to 11 years). Anthropometric measures (weight, height, head circumference, and BMI) varied by patient. There were no significant changes in measurement values or percentiles from the beginning to the end of the study. BMI improved in all 3 patients during the study, with percentiles that increased in two patients who were underweight at the beginning of the study (from the 22^{nd} to the 50^{th} and from the 3^{rd} to the 46^{th} percentile, respectively) and decreased in a third patient who was overweight (from the 95^{th} to the 91^{st} percentile), though these improvements were not statistically significant.

Table 2 reports levels of hemoglobin, white blood cell counts, platelets, transaminases, alkaline phosphatase, total proteins and albumin at baseline and during treatment with ornithine α-ketoglutarate, glutamine or citrate. There were no statistically significant differences between average biochemical baseline values and averages from each of the three treatment periods for the lab values reported (Table 2). Patients also had normal free thyroxine and thyroid stimulating hormone when measured at baseline and upon completion. Amylase and lipase remained within normal limits throughout the

Table 1

Demographic Information

	Patient	1	2	3	
	Gender Race	F Hispanic	F White	M White	Average:
Age (yr)	Before	5.9	11	6.8	7.9 ±2.7
	After	7.3	12.2	7.8	9.1±2.7
Weight, kg (centile)	Before	15.6 (2)	45.2 (80)	20.2 (18)	27±15.9 (33±41)
	After	19.7 (9)	48.9 (74)	27.2 (67)	31.9±15.2 (50±36)
Height, cm (centile)	Before	104.5 (2)	136.5 (14)	122 (57)	120.5±22.6 (24±29)
	After	112.5 (1)	143.3 (10)	132 (80)	127.9±21.8 (30±43)
Head circumference, cm (centile)	Before	47.2 (<3)	51 (10)	49.5 (3)	49.2 ± 1.9
	After	48 (<3)	51.5 (10)	50 (3)	49.8±1.8
Body mass Index, kg/m^2 (centile)	Before	14.3 (22)	24.3 (95)	13.6 (3)	17.4 ± 6 (40±48.6)
	After	15.6 (50)	23.8 (91)	15.6 (46)	18.3±4.7 (62±25)

Demographic information in patients with propionic acidemia. Measurements taken at baseline and at the conclusion of the study period. Results reported as mean ± SD.

Table 2

Safety Labs

Labs:	Normal	Baseline	Citrate	Glutamine	OKG
Hemoglobin (g/dL)	11-13.3	13.1± 2.1	12.8±1.4	12.6±1.1	12.1±1.0
White Blood Cells (k/uL)	4.5-10.5	5.3±1.5	5.2±1.8	6.0±2.5	5.4±1.0
Platelets (k/uL)	204-405	234±70	233±70	273±90	302±94
AST (U/L)	20-60	60.3±24.4	59.9±22.4	47.9±11.4	44.9±6.7
ALT (U/L)	5-45	45.8±29.6	54.2±32.4	33.5±15.7	35.4±11.5
Alk. Phos (U/L)	145-320	189±57	207±44	191±53	175±51
Total Protein (g/dL)	5.9-7.0	6.7±0.7	6.6±0.5	6.9±0.6	6.7±0.7
Albumin (g/dL)	3.1-4.2	3.9±0.4	3.8±0.2	3.9±0.3	4.0±0.3

Safety Labs in patients with propionic acidemia. Values are averages ± SD of at least 12 observations. OKG: Ornithine α-ketoglutarate.

study. Serum lactate levels also did not change significantly during the study, with very few values above the normal range. As noted in Figure 3A, ammonia levels did not change significantly during the trial as did the urinary excretion of lactate and pyruvate (Figure 3B). Overall, routine lab values were not significantly affected by any of the treatments indicating overall safety of the supplements.

Amino Acids and Organic Acids

In the first part of the study, patients with propionic acidemia received three different supplements (ornithine α-ketoglutarate, glutamine and citrate) for 4 weeks with blood testing every week. Values obtained in the presence of supplements were compared to baseline (before the initiation of the study and during wash-out periods). None of the treatments significantly increased [glutamine] or [glutamine] + [glutamate] concentration nor significantly decreased plasma ammonia (Figure 3A). For this reason, we looked at the urinary excretion of Krebs cycle intermediates (urine organic acids) for evidence that the supplements entered mitochondria. Levels of ketoglutarate did not increase significantly during treatment with ornithine α-ketoglutarate or glutamine, but did increase significantly during citrate therapy ($p<0.01$) (Figure 3B). Levels of fumarate and succinate also increased significantly during citrate treatment compared to baseline ($p<0.001$), but not with the two other supplements. Levels of lactate, pyruvate, and citrate also increased during citrate treatment, though this increase was not statistically significant compared to baseline. In summary, citrate, but not glutamine nor ornithine α-ketoglutarate significantly increased the urinary excretion of ketoglutarate, succinate and fumarate, all metabolites produced after citrate entry into mitochondria and its

modification by enzymes of the Krebs cycle (Figure 3B, 3C). At the same time, there was no statistically significant increase in the excretion of lactate and pyruvate, which accumulate when the Krebs cycle is dysfunctional. Thus, citrate was supplemented in the second part of the study for 30 weeks.

To evaluate changes in plasma amino acids, the results of both parts of the study were grouped together and are summarized in Tables 3 and 4 and Figure 3. Table 3 shows the average values of the 22 amino acids collected at baseline and during each treatment period. The majority of amino acids remained unchanged throughout the study. As expected, ornithine levels increased significantly ($p<0.001$ compared to baseline) during ornithine α-ketoglutarate supplementation. This indicated that the chemical was successfully absorbed in the gut. However, ketoglutarate excretion in urine did not increase during the ornithine α-ketoglutarate treatment period (Figure 3B) indicating that α-ketoglutarate was either metabolized prior to entry into mitochondria or did not enter the Krebs cycle in sufficient amounts to increase its urinary excretion or that of downstream metabolites.

Leucine, phenylalanine and threonine decreased significantly compared to baseline (Table 3) during supplementation with ornithine α-ketoglutarate ($p<0.01$, $p=0.014$, and $p=0.037$, respectively), possibly due to increased restriction of natural proteins and use of formula not containing threonine. The only amino acid that changed significantly ($p=0.02$) during citrate supplementation was leucine that decreased about 15% below baseline (Table 3). This might have also been due to mildly increased dietary restriction of natural proteins.

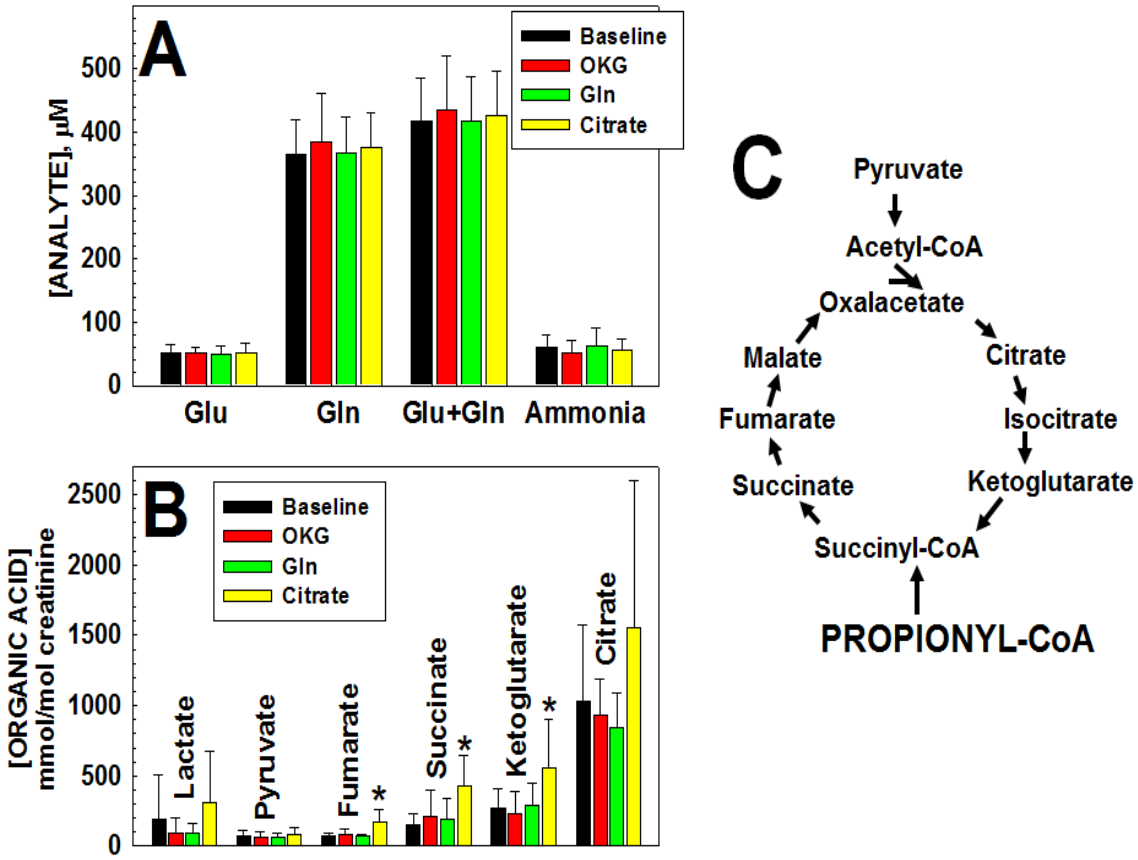

Figure 3. Effect of anaplerotic therapy on selected plasma amino acids, ammonia, and urine organic acids. Effect of anaplerotic therapy on (A) select amino acids, ammonia and (B) urinary organic acids in patients with propionic acidemia. Each value is the average ± SD of at least 12 observations. (C) Schematic of the Krebs cycle.
*P< 0.05 (or better) versus baseline.

Table 3

Plasma Amino Acids

Amino Acid	Normal	Baseline	Citrate	Glutamine	OKG
Alanine	240-600	349.1 ± 84.5	330.9 ± 91.2	328 ± 79	362.3 ± 64.8
Arginine	40-160	42.8 ± 11.7	36.5 ± 10.2	45.3 ± 21.4	41.4 ± 10.9
Asparagine	15-40	31.2 ± 13.1	27.6 ± 9.9	26 ± 9.5	29.2 ± 5.0
Aspartate	0-20	11.1 ± 4.6	9.9 ± 2.8	9.7 ± 2.5	11.7 ± 2.8
Citrulline	10-60	19.58 ± 4.21	17.5 ± 4.9	21.4 ± 7.9	21.9 ± 4.8
Cysteine	7-70	34 ± 7.5	29.7 ± 6.9	33.5 ± 7.1	32.1 ± 7
Glutamine	410-700	365.3 ± 54.5	374.7 ± 49.1	367.3 ± 55.3	390.9 ± 73.7
Glutamate	10-120	51.2 ± 14.2	50.7 ± 15.6	49.7 ± 12.6	52 ± 7.3
Glycine	140-490	1268.3 ± 176.6	1173 ± 244	1248.8 ± 229.1	1376.1 ± 157.9
Histidine	50-130	76.9 ± 20.3	67.7 ± 22.1	60.8 ± 20.2	71.9 ± 20.4
Isoleucine	30-130	20.7 ± 10.6	20.6 ± 9.5	20.7 ± 11	23.3 ± 9.5
Leucine	60-230	69.5 ± 35.7	60.8 ± 21.7*	64.3 ± 22.6	50.1 ± 15.8
Lysine	80-250	227.3 ± 76.1	225.8 ± 68.3	243.9 ± 102.8	224.3 ± 82.2
Methionine	17-53	12.6 ± 3	13.5 ± 3.6	13.9 ± 3.9	12.7 ± 2.9
Ornithine	20-135	40.7 ± 16.9	32.6 ± 16	37.3 ± 12.7	94.3 ± 39.2*
Phenylalanine	30-80	42.6 ± 9.5	41 ± 7	39.4 ± 7.9	34.3 ± 5.2*
Proline	110-500	252.0 ± 92	307.8 ± 78.5	264.1 ± 64.3	283.8 ± 70.2
Serine	60-200	142.4 ± 21.7	131.7 ± 28.5	131.7 ± 32.7	147 ± 17
Taurine	25-80	67.8 ± 15.6	60.8 ± 8.8	61.2 ± 7.1	66.5 ± 10.2
Threonine	60-220	65.3 ± 15.2	74 ± 26	55.4 ± 14.2	54.1 ± 8.7*
Tyrosine	30-120	79.8 ± 20.8	67.4 ± 20	80.1 ± 21	66.1 ± 17.6
Valine	140-350	60.7 ± 25.6	67.8 ± 28.2	64 ± 26.6	55.5 ± 24.8

Plasma Amino Acids (μmol/L) in patients with propionic acidemia. Values are averages ± SD of at least 12 observations.
*$p < 0.05$ (or better) versus baseline

Table 4

Correlation of Ammonia Levels with Plasma Amino Acids

Amino Acid	R^2	Significance P
Alanine	0.07*	0.041
Arginine	0.29*	<0.001
Asparagine	0.01	0.380
Citrulline	0.13*	0.006
Cysteine	0.01	0.388
Glutamine	0.02	0.282
Glutamine + Glutamate	0.05	0.112
Glutamine + Glutamate + Alanine	0.09*	0.026
Glutamate	0.12*	0.008
Glycine	0.06	0.074
Histidine	0.02	0.240
Isoleucine	0.01	0.369
Leucine	0.11*	0.012
Lysine	0.52*	<0.001
Methionine	0.02	0.278
Ornithine	0.05	0.108
Phenylalanine	0.10*	0.014
Proline	0.006	0.582
Serine	0.02	0.246
Threonine	0.04	0.148
Tyrosine	0.13*	0.007
Valine	0.005	0.608

Correlation of ammonia levels with plasma amino acids in patients with propionic acidemia during the clinical trial. Statistical significance was calculated using analysis of variance. R2 and p are indicated.
*p <0.05 or better

In propionic acidemia, glutamine levels are low even in well-controlled patients and decrease (rather than increase) with hyperammonemia (12). This might be due to low levels of α -ketoglutarate favoring release of nitrogen from glutamine and glutamate to generate the Krebs cycle intermediate (12). Since all supplements used in the study could have potentially increased α -ketoglutarate and reversed this relationship, we explored whether the negative correlation between ammonia and glutamine/glutamate and alanine were still observed during the study. As shown in Figure 4 and Table 4, glutamine levels did not decrease with increasing ammonia levels (panel B) and levels of glutamate (A) and alanine (C) significantly increased, rather than decreased with increasing ammonia levels. This suggests that these therapies might have re-established, at least in part, ketoglutarate levels and the physiological mechanism of ammonia buffering by the centrilobular hepatocytes.

In propionic acidemia, levels of ammonia positively correlated with an increase in the amino acids leucine, tyrosine and phenylalanine (12). During this clinical trial we observed the same trend (Table 4, Figure 5C, E, F). Since these amino acids are essential and provided both by natural proteins and the special formula that patients with propionic acidemia consume, these results suggest that ammonia levels correlated to overall protein intake. In addition to these large neutral amino acids, arginine, citrulline, and lysine increased with ammonia levels (Figure 5A, B, D), with lysine rising in many occasions above the normal range (Figure 5D). There was no significant correlation between ammonia levels and the remaining amino acids (Table 4).

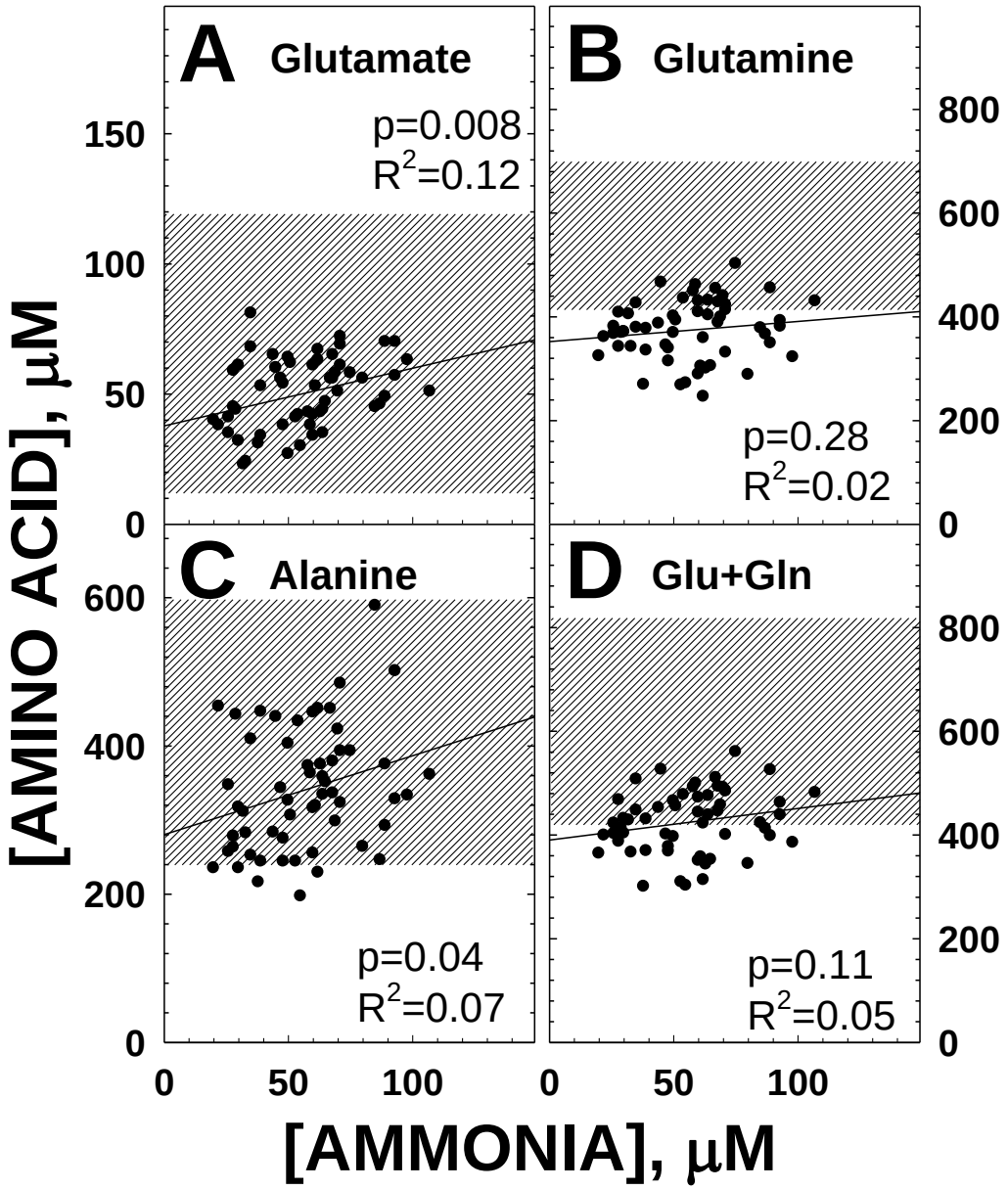

Figure 4. Correlation of ammonia levels with selected plasma amino acids. Correlation of ammonia levels with select plasma amino acids in patients with propionic acidemia during the clinical trial. Data were analyzed by linear regression Results for each amino acid are indicated in the figure. The shaded area represents the normal range of variation.

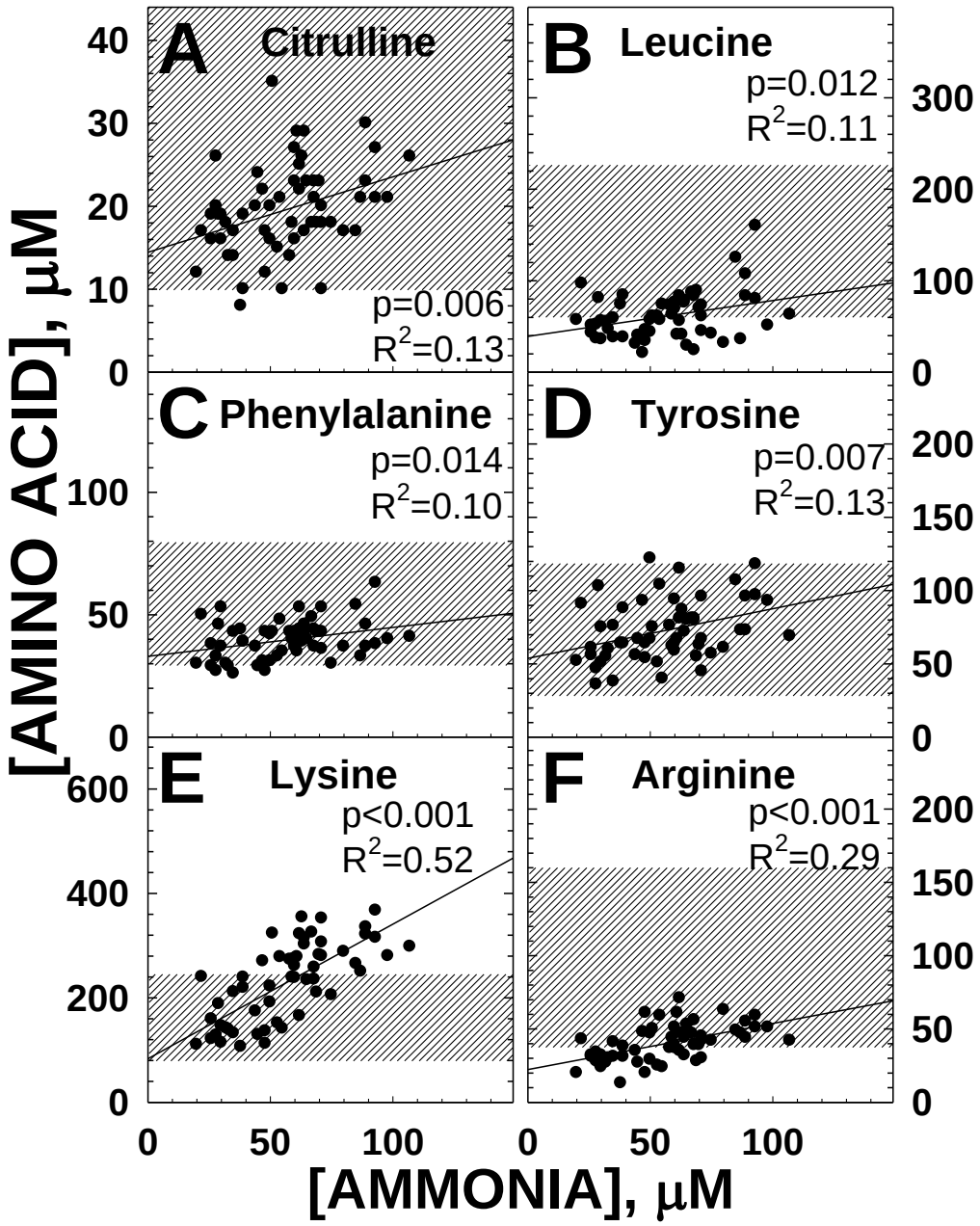

Figure 5. Correlation of ammonia levels with selected plasma amino acids. Correlation of ammonia levels with selected plasma amino acids in patients with propionic acidemia during the clinical trial. Data were analyzed by linear regression Results for each amino acid are indicated in the figure. The shaded area represents the normal range of variation. Note that several lysine levels were above the normal range.

Cognitive and Motor Assessment

The cognitive assessment (Table 5) indicated that all patients had severely compromised intellectual abilities before the study. There were no changes in nonverbal IQ, verbal IQ, or full scale IQ from the beginning to the end of the study in patients 1 or 2. Patient 3 was too severely affected and unable to complete the evaluation at the beginning and end of the study.

Table 6 shows the results from the motor assessment of patients 1 and 2. Patient 3 was unable to perform the tasks of the assessment. The hand-held myometry composite score increased for both patient 1 and 2 during the course of the study. Results of the time function tests were variable between the patients. Time required to walk/run 10 meters and to climb 4 stairs improved for patient 1, but the time to rise from supine to stand worsened. Patient 2 was unable to complete the 10 meter walk/run and the rise from supine to stand test because she did not have her walker during the final day of testing. The time to climb four stairs worsened for patient 2 during the study. Accelerometry studies showed that both patient 1 and 2 became less active from the beginning to the end of the study with decreases in average steps in 7 days, average minutes of activity in 7 days, and increase in average % of inactivity in 7 days.

Hospitalizations

To evaluate the effect of treatment on overall health, we counted the number of days that our patients spent in the hospital before, during and after the clinical trial while they were continuing on the supplements (Table 7). Hospitalization days were normalized per year to account for the shorter time span during the study compared to the 2 years

Table 5

Cognitive Assessment

	Nonverbal IQ		Verbal IQ		Full Scale IQ	
Pt.	Before (Percentile)	After (Percentile)	Before (Percentile)	After (Percentile)	Before (Percentile)	After (Percentile)
1	42 (<0.1)	42 (<0.1)	43 (<0.1)	43 (<0.1)	40 (<0.1)	40 (<0.1)
2	42 (<0.1)	42 (<0.1)	43 (<0.1)	43 (<0.1)	40 (<0.1)	40 (<0.1)
3	Child not sufficiently cooperative					
Average:	42 (<0.1)	42 (<0.1)	43 (<0.1)	43 (<0.1)	40 (<0.1)	40 (<0.1)

Cognitive Assessment using the Stanford Binet Fifth Edition in patients with propionic acidemia.

Table 6

Motor Assessment

		Pt:	1	2
Hand-Held Myometry	Composite Score (lbs)	Before	10.85	12.6
		After	14.7	12.85
Time Function Test	10 meter walk/run (sec)	Before	7.1	14.6
		After	5	NA**
	Time to rise from supine to stand (sec)	Before	3.3	NA**
		After	4.2	NA**
	Time to climb 4 stairs (sec)	Before	4.3	12.9
		After	2.7	14.7
Accelerometry Tests	Average steps in 7 days	Before	7679	2192
		After	5599	1891
	Average minutes of activity in 7 days	Before	496	247
		After	485	227
	Average % of inactivity in 7 days	Before	65.5	82.8
		After	66.3	84.21

Evaluation of muscle activity in patients with propionic acidemia. Results reported at baseline and at the conclusion of the study.
*Patient 3 was unable to complete the evaluation of muscle activity.
** Patient 2 did not bring her walker on the final day of testing and was unable to perform these tasks.

Table 7

Hospitalizations

	Two years prior to study		During study		Two years following study	
Pt:	Days Hospitalized	ER visits	Days Hospitalized	ER visits	Days Hospitalized	ER visits
1	19	3.5	10	2.1	4.5	0.5
2	25	0.5	32	0	0	0.5
3	2	0	5.5	0	1	0
Average± SD	15.3±11.9	1.3±1.9	15.8±14.2	0.7±1.2	1.8±2.4*	0.3±0.3
Total:	46	4	47.5	2.1	5.5	1

Hospitalizations in patients with propionic acidemia before, during and after anaplerotic therapy. Average number of days hospitalized and ER visits in patients with propionic acidemia two years prior to study, during study, and two years following study. The number of days spent in the hospital was normalized per year.
*p <0.05 versus prior to study using analysis of variance.

before and after the study. The cause of hospitalizations was related mostly to respiratory problems, pancreatitis, or viral infections. In no case were adverse events deemed related to treatment, but rather to the underlying medical condition or intercurrent illnesses. Patients with propionic acidemia did not have any change in the number of days in the hospital during the study. However, the average number of days/year that patients were hospitalized significantly decreased during the 2 years after the study, while the patients continued to supplement with open-label citrate, as compared to the 2 years prior to the study ($p=0.04$) or during the study ($p=0.03$). This suggests that citrate therapy might be clinically effective, but might require prolonged time before generating measurable changes. The number of ER visits not leading to hospital admissions during and after the study was also lower than the number of ER visits per year during the 2 years prior to the study, though the changes were not statistically significant.

DISCUSSION

The objective of this study was to determine whether citrate, glutamine, or ornithine α-ketoglutarate were effective in raising plasma glutamine and reducing plasma ammonia levels compared to baseline in patients with propionic acidemia. All supplements were safe, with no adverse events attributed to them nor changes in safety laboratory values (Table 2). Growth remained appropriate during the study. BMI (kg/m^2) increased in patients 1 and 3, who started with a BMI lower than average, and decreased in patient 2, who started with an above average BMI (Table 1). Overall, there were no indications that any of the supplements were detrimental to the patients' health.

In the first part of the study, none of the supplements significantly raised the plasma glutamine or [glutamine+glutamate] concentration (Figure 3A). Ammonia levels also did not change significantly during therapy with any of the 3 supplements. Despite lack of significant changes in glutamine levels, urine organic acids indicated that treatment with citrate, but not the other supplements, significantly increased the excretion of ketoglutarate, succinate and fumarate (Krebs cycle intermediates, Figure 3B, C), indicating that citrate entered mitochondria and was converted to subsequent intermediates in the Krebs cycle possibly providing successful anaplerotic therapy. For this reason, treatment with citrate was continued over time (30 weeks) to determine its effects on clinical outcomes and biochemical parameters.

Even with a longer period of time on citrate, therapy failed to significantly increase glutamate or glutamine levels (Table 3). The only changes that were observed in the plasma amino acids were an increase in ornithine in patients receiving ornithine α-ketoglutarate and a reduction in some essential amino acids, possibly reflecting more strict dietary compliance. With respect to plasma ammonia, the inverse relationship between plasma ammonia and glutamine levels (12) was lost during the trial (Figure 4), suggesting that more substrate (ketoglutarate) may have been available to hepatocytes to conjugate ammonia escaping the urea cycle in periportal hepatocytes. At the same time, there was a striking direct correlation between ammonia and lysine levels (R^2=0.52, $p<0.0001$) (Figure 5). Lysine levels increase in many forms of hyperammonemia and in urea cycle defects. Since lysine is an essential amino acid, its increased levels likely reflect decreased degradation. There are two pathways devoted to lysine breakdown: the saccharopine and the pipecolic acid routes that ultimately converge at the level of al α-aminoadipic semialdehyde. In the saccharopine pathway, that is believed to be the main catabolic route, L-lysine is converted to saccharopine by α-aminoadipic semialdehyde synthase by condensation with α-ketoglutaric acid (35, Figure 6). In the pipecolic acid pathway, transamination also requires an acceptor for ammonia that, in most cases, is α -ketoglutarate. In urea cycle defects, ammonia combines with α-ketoglutarate to generate glutamate and then glutamine, leading to α-ketoglutarate depletion and a secondary increase in lysine levels. In propionic acidemia, it would be the primary depletion of α-ketoglutarate that results in a stable increase in lysine levels, which further increase when residual α-ketoglutarate is conjugated with ammonia to generate glutamic acid. If this is the mechanism, normalization of lysine levels would become a further indicator of

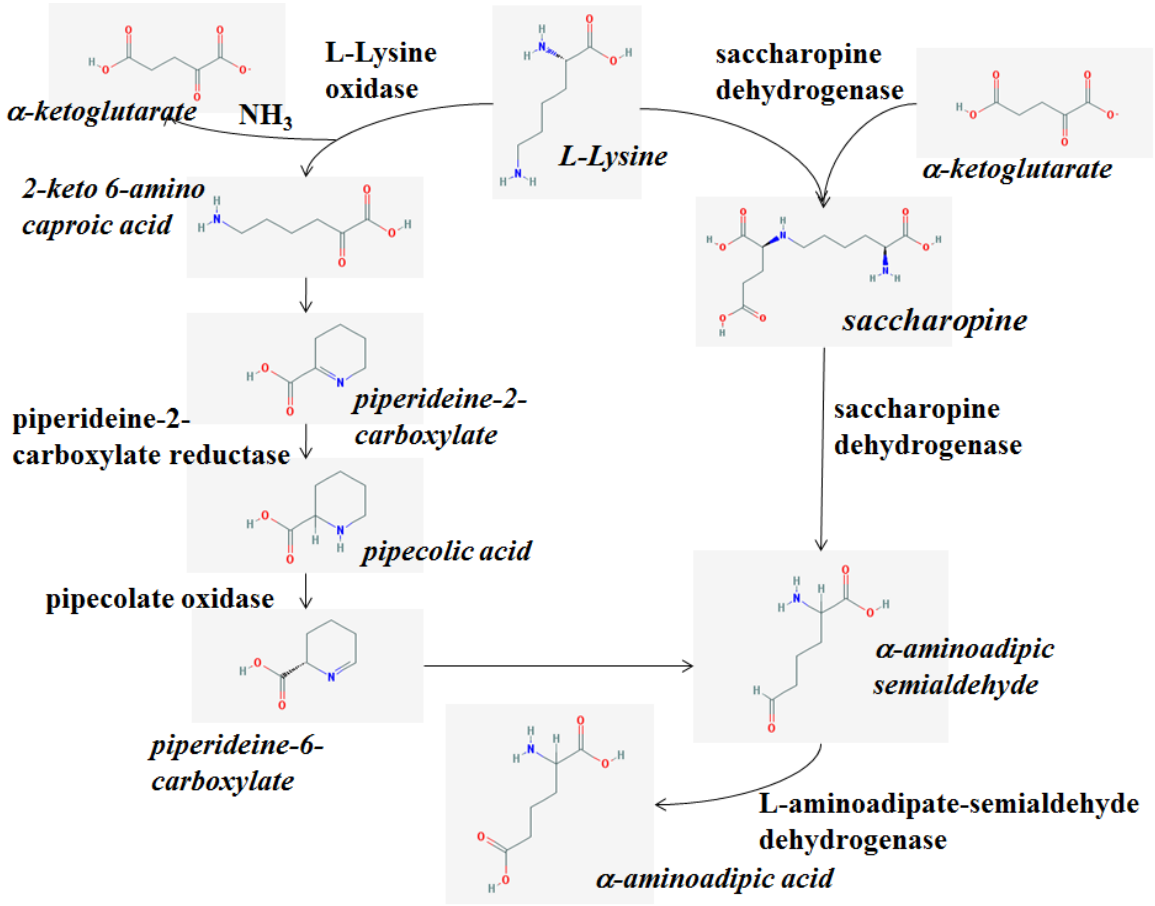

Figure 6. Schematic of lysine degradation pathways.
Note that both pathways require availability of α-ketoglutarate.

successful therapy in patients with propionic acidemia. In addition, mild restriction of dietary lysine could spare ketoglutarate for anaplerotic functions.

Most patients with propionic acidemia have developmental delays. We tested the effects of supplements on cognitive and motor delays. Only two patients were able to participate in the motor assessment, since the third one was too impaired to perform the required tasks. The small sample size precluded any statistical analysis. Cognitive function, assessed with the Stanford Binet method, was severely impaired in our patients with propionic acidemia (average IQ=40) and did not improve with treatment (Table 5). This was not unexpected since all of our patients had a severe initial presentation that caused brain suffering not usually responsive to therapy.

Hand-held myometry, a test of muscle strength, increased in both tested patients from the beginning to the end of the study (Table 6). No consistent changes were measured in time function tests (time required to walk/run 10 meters, to rise from supine to stand, and to climb four stairs) and in the accelerometry tests. The latter test showed a decline in activity in both patients from the beginning to the end of the study. There are several factors that may have influenced the activity level of patients at the beginning and end of the study. The decrease in activity may be due to differences in season/circumstances, from the beginning to the end of the study, rather than a decreased ability to be physically active. These patients also had motor delays prior to beginning the study and, overall, were less able to understand and complete the motor evaluation tests successfully compared to the general population. Overall, treatment with citrate did not affect cognitive function and was, at best, moderately effective in only one test of motor function.

While there was no effect on cognitive or motor development, the overall health of patients with propionic acidemia might have benefitted from citrate supplementation. In the 2 years prior to the study, patients were hospitalized 15.3 days per year on average (Table 7). During the study, the number remained at 15.8 days per year. In the 2 years poststudy, all three patients remained on open label citrate supplements and the average number of days hospitalized per year decreased significantly to 1.8 ($p=0.04$ as compared to baseline). The significant reduction in the number of hospitalization days during this time suggests that citrate supplements might improve the general health of patients with propionic acidemia, but that the effects of supplements might require time to become evident. The decreased number of hospitalizations could be due to factors other than therapy. In general, patients with propionic acidemia become more clinically stable as they get older. However, the patient who improved the most (patient 2) in this case was the oldest of the group and was already 11 years old when the study started. All of the patients improved regardless of age. Patients and their families also had extended contacts with the healthcare team consisting of physicians and dietitians, during the study. Improved education about the disease and consistent monitoring could have helped the management of propionic acidemia in the long term.

There are several limitations in our study. Our study involved only three patients with propionic acidemia, a rare metabolic disorder, affecting approximately 1:50,000 worldwide (36). The results of this pilot study results should be extended to a larger population of patients with a multicenter trial enabling enrollment of an adequate number of patients.

Another factor that may have affected the results of the study may be the dose of the supplement. On a molar basis, OKG dosing provided 2 mmol/kg ornithine, 1 mmol/kg α-ketoglutarate, glutamine was supplemented at 2.7 mmol/kg, and citrate was supplemented at 2.5 mmol/kg. Therefore, considering that ornithine can be converted into ketoglutarate (Figure 2), the dosage of the supplements was almost equivalent. However, these dosing levels may have not been high enough to cause glutamine levels to rise. Specifically, the dose of citrate might have increased intermediates of the citric acid cycle, but might have not been sufficiently high to increase glutamate and glutamine levels. For this reason, higher doses of citrate should be tested in future patients.

CONCLUSION

Propionic acidemia is a rare metabolic disorder caused by deficiency of propionyl-CoA carboxylase. Patients with propionic acidemia generally have a poor prognosis due to health complications including pancreatitis or cardiomyopathy. Deficient breakdown of propionyl-CoA may lead to deficient levels of Krebs cycle intermediates and decreased glutamine levels. Current treatment in patients with propionic acidemia includes a low protein diet with an artificial formula that does not contain the amino acids threonine, valine, leucine, and methionine. Patients receive carnitine supplements to replace propionylcarnitine that is lost in urine. Adding a supplement to their treatment regimen would be a simple, effective way to improve the prognosis.

By supplementing citrate in patients with propionic acidemia, we have shown their ability to increase Krebs cycle intermediates through anaplerosis and (for all supplements) to normalize the relationship between ammonia and glutamate levels. This treatment appears to be safe and effective in the long term in reducing hospitalizations in patients with propionic acidemia. Further testing of citrate therapy at a higher dose on a larger population might prove useful not only in treating patients with propionic acidemia, but also patients with other organic acidemias in which there is depletion of Krebs cycle intermediates.

REFERENCES

1. Fenton W, Gravel, RA, Rosenblatt, DS. Disorders of propionate and methylmalonate metabolism. In: *The Metabolic and Molecular Bases of Inherited Disease*, edited by Scriver C, Beaudet, AL, Sly, WS, Valle, D. New York: McGraw-Hill, 2001, p. 2165-2194.

2. Ierardi-Curto L, Kaplan P, Saitta S, Mazur A, and Berry GT. The glutamine paradox in a neonate with propionic acidaemia and severe hyperammonaemia. *J Inherit Metab Dis* 2000; 23: 85-86.

3. Deodato F, Boenzi S, Santorelli FM, and Dionisi-Vici C. Methylmalonic and propionic aciduria. *Am J Med Genet C Semin Med Genet* 2006; 142: 104-112.

4. Dionisi-Vici C DF, Röschinger W, Rhead WJ, Wilcken B. "Classical" Organic Acidurias, Propionic- Methylmalonic- and Isovaleric-aciduria, long-term outcome and effects of expanded newborn screening using tandem mass spectrometry. *J Inerit Metab Dis* 2006; 29: 383-389.

5. Coude FX, Sweetman L, and Nyhan WL. Inhibition by propionyl-coenzyme A of N-acetylglutamate synthetase in rat liver mitochondria. A possible explanation for hyperammonemia in propionic and methylmalonic acidemia. *J Clin Invest* 1979; 64: 1544-1551.

6. Stewart PM and Walser M. Failure of the normal ureagenic response to amino acids in organic acid-loaded rats. Proposed mechanism for the hyperammonemia of propionic and methylmalonic acidemia. *J Clin Invest* 1980; 66: 484-492.

7. Gebhardt B, Dittrich S, Parbel S, Vlaho S, Matsika O, and Bohles H. N-carbamylglutamate protects patients with decompensated propionic aciduria from hyperammonaemia. *J Inherit Metab Dis* 2005; 28: 241-244.

8. Gebhardt B, Vlaho S, Fischer D, Sewell A, and Bohles H. N-carbamylglutamate enhances ammonia detoxification in a patient with decompensated methylmalonic aciduria. *Mol Genet Metab* 2003; 79: 303-304.

9. Al-Hassnan ZN, Boyadjiev SA, Praphanphoj V, Hamosh A, Braverman NE, Thomas GH, and Geraghty MT. The relationship of plasma glutamine to ammonium and

of glycine to acid-base balance in propionic acidaemia. *J Inherit Metab Dis* 2003; 26: 89-91.

10. Tuchman M and Yudkoff M. Blood levels of ammonia and nitrogen scavenging amino acids in patients with inherited hyperammonemia. *Mol Genet Metab* 1999; 66: 10-15.

11. Tuchman M, Lichtenstein GR, Rajagopal BS, McCann MT, Furth EE, Bavaria J, Kaplan PB, Gibson JB, and Berry GT. Hepatic glutamine synthetase deficiency in fatal hyperammonemia after lung transplantation. *Ann Intern Med* 1997; 127: 446-449.

12. Filipowicz HR, Ernst S, Ashurst CL, Pasquali M, Longo N. Metabolic changes associated with hypermmonemia in patients with propionic acidemia. *Molecular Genetics and Metabolism* 2006; 88: 123-130.

13. Owen OE, Kalhan SC, and Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. *J Biol Chem* 2002; 277: 30409-30412.

14. Mochel F, DeLonlay P, Touati G, Brunengraber H, Kinman RP, Rabier D, Roe CR, and Saudubray JM. Pyruvate carboxylase deficiency: clinical and biochemical response to anaplerotic diet therapy. *Mol Genet Metab* 2005; 84: 305-312.

15. Roe CR, Sweetman L, Roe DS, David F, and Brunengraber H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. *J Clin Invest* 2002; 110: 259-269.

16. Gibala MJ, MacLean DA, Graham TE, and Saltin B. Anaplerotic processes in human skeletal muscle during brief dynamic exercise. *J Physiol* 1997; 502 (Pt 3): 703-713.

17. Brunengraber H and Roe CR. Anaplerotic molecules: Current and future. *J Inherit Metab Dis* 2006; 29: 327-331.

18. Iafolla AK, Gale DS, and Roe CR. Citrate therapy in argininosuccinate lyase deficiency. *J Pediatr* 1990; 117: 102-105.

19. Renner C, Sewell AC, Bervoets K, Forster H, and Bohles H. Sodium citrate supplementation in inborn argininosuccinate lyase deficiency: a study in a 5-year-old patient under total parenteral nutrition. *Eur J Pediatr* 1995; 154: 909-914.

20. Ahmad A, Kahler SG, Kishnani PS, Artigas-Lopez M, Pappu AS, Steiner R, Millington DS, and Van Hove JL. Treatment of pyruvate carboxylase deficiency with high doses of citrate and aspartate. *Am J Med Genet* 1999; 87: 331-338.

21. Matthews DE, Marano MA, and Campbell RG. Splanchnic bed utilization of glutamine and glutamic acid in humans. *Am J Physiol* 1993; 264: E848-854.

22. Kalhan SC, Parimi PS, Gruca LL, and Hanson RW. Glutamine supplement with parenteral nutrition decreases whole body proteolysis in low birth weight infants. *J Pediatr* 2005; 146: 642-647.

23. Aquino VM, Harvey AR, Garvin JH, Godder KT, Nieder ML, Adams RH, Jackson GB, and Sandler ES. A double-blind randomized placebo-controlled study of oral glutamine in the prevention of mucositis in children undergoing hematopoietic stem cell transplantation: a pediatric blood and marrow transplant consortium study. *Bone Marrow Transplant* 2005; 36: 611-616.

24. Cynober L, Coudray-Lucas C, de Bandt JP, Guechot J, Aussel C, Salvucci M, and Giboudeau J. Action of ornithine alpha-ketoglutarate, ornithine hydrochloride, and calcium alpha-ketoglutarate on plasma amino acid and hormonal patterns in healthy subjects. *J Am Coll Nutr* 1990; 9: 2-12.

25. Cynober LA. The use of alpha-ketoglutarate salts in clinical nutrition and metabolic care. *Curr Opin Clin Nutr Metab Care* 1999; 2: 33-37.

26. Coudray-Lucas C, Le Bever H, Cynober L, De Bandt JP, and Carsin H. Ornithine alpha-ketoglutarate improves wound healing in severe burn patients: a prospective randomized double-blind trial versus isonitrogenous controls. *Crit Care Med* 2000; 28: 1772-1776.

27. Moukarzel AA, Goulet O, Salas JS, Marti-Henneberg C, Buchman AL, Cynober L, Rappaport R, and Ricour C. Growth retardation in children receiving long-term total parenteral nutrition: effects of ornithine alpha-ketoglutarate. *Am J Clin Nutr* 1994; 60: 408-413.

28. Miyazaki T, Ohura T, Kobayashi M, Shigematsu Y, Yamaguchi S, Suzuki Y, Hata I, Aoki Y, Yang X, Minjares C, Haruta I, Uto H, Ito Y, and Muller U. Fatal propionic acidemia in mice lacking propionyl-CoA carboxylase and its rescue by postnatal, liver-specific supplementation via a transgene. *J Biol Chem* 2001; 276: 35995-35999.

29. Moller SE. Quantification of physiological amino acids by gradient ion-exchange high-performance liquid chromatography. *J Chromatogr* 1993; 613: 223-230.

30. Tanaka K, West-Dull A, Hine DG, Lynn TB, and Lowe T. Gas-chromatographic method of analysis for urinary organic acids. II. Description of the procedure, and its application to diagnosis of patients with organic acidurias. *Clin Chem* 1980; 26: 1847-1853.

31. Rabier D, Parvy P, Bardet J, and Kamoun P. [Hyperlysinemia and hyperammonemia]. *Ann Biol Clin (Paris)* 1991; 49: 45-48.

32. Krieger I, Bachmann C, Gronemeyer WH, and Cejka J. Propionic acidemia and hyperlysinemia in a case with ornithine transcarbamylase (OTC) deficiency. *J Clin Endocrinol Metab* 1976; 43: 796-802.

33. Lehnert W, Sperl W, Suormala T, and Baumgartner ER. Propionic acidaemia: clinical, biochemical and therapeutic aspects. Experience in 30 patients. *Eur J Pediatr* 1994: 153: S68-80.

34. Chandler RJ, Chandrasekaran S, Carrillo-Carrasco N, Senac JS, HOfherr SE, Barry MA, Venditti CP. Adeno-associated virus serotype 8 gene transfer rescues neonatal lethal murine model of propionic acidemia. *Hum Gene Ther* 2011; 22(4): 477-81.

35. Dancis J, Hutzler J, Woody NC, Cox RP. Multiple enzyme defects in familial hyperlysinemia. *Pediatr. Res*. 1997; 10: 686–691.

36. Desviat LR, Pérez B, Pérez-Cerdá C, Rodríguez-Pombo P, Clavero S, Ugarte M. Propionic Acidemia: mutation update and functional and structural effects of variant alleles. *Mol Genet Metab. 2004;* 83:28-37.